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DIFFERENTIAL *IN VIVO* SENSITIVITY OF MELANOPHORES AND XANTHOPHORES TO CATECHOLAMINES IN WINTER FLOUNDER (*PSEUDOPLEURONECTES AMERICANUS* WALBAUM) INTEGUMENTARY PATTERNS

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SUMMARY

The catecholamines, adrenalin, dopamine and noradrenalin induce differential aggregation of melanophores in black-adapted winter flounder, *Pseudopleuronectes americanus*, paralleling patterning responses to albedo change. These differential responses to catecholamines suggest that the patterning mechanism in this species is largely dependent on a balance between neural aggregating and dispersing elements. The α-adrenoceptor agonist phenylephrine evokes paling in all pattern components in black-adapted flounder, whilst the α-adrenoceptor antagonist phentolamine darkens white-adapted flounders. The α-adrenoceptor agonist isoproterenol and the α-adrenoceptor antagonist propranalol have no effect on chromatophores of white-adapted flounder, but induce pallor in black-adapted flounder, which is interpreted as non specific. Noradrenalin elicits patterning responses in chromatically decentralized flounder, although the duration of pallor is shorter. The xanthophores, which are dispersed by a pituitary factor, will aggregate in response to high catecholamine doses.

INTRODUCTION

The dark upper surface of winter flounder (*Pseudopleuronectes americanus*) has three major pattern components (dark bands, a general background component and pale iridophore spots) with different relative capacities of their dermal and epidermal melanophores (DM, EPM) and xanthophores (XA) for pigment movement in response to albedo change (Burton, 1981). The DM and EPM, with dark masking pigments and neurally controlled differential responses, predominate in patterning. Band melanosomes disperse rapidly but aggregate slowly, whilst general background and iridophore spot melanosomes aggregate rapidly but disperse slowly. The bands also exhibit stress-related darkening and the prominence of the iridophore spots is not entirely background related. The sympathetic innervation of teleost DM, promoting melanosome aggregation, is well established (Bagnara &

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Hadley, 1973), and the catecholaminergic nature of this innervation has been demonstrated by histochemical fluorescence (Jacobowitz & Laties, 1968). Differential patterning responses of flounder melanophores suggest differences in balance between melanosome aggregating and dispersing neurones and/or neurotransmitter receptor types. The main purpose of the present work was to determine whether there are pattern-related differences in catecholaminergic sensitivities, which would support the view that control is mediated through opposing neural elements.

Identification of individual catecholamine (CA) chromatophore neurotransmitter compounds has not been achieved, although noradrenalin (NA) is considered the most likely (Visconti & Castrucci, 1981). Adrenalin (A) and dopamine (DA), also induce teleost melanosome aggregation, and in *Pleuronectes platessa* DA has been found to be the most effective both *in vivo* and *in vitro* (Fernando & Grove, 1974*a*,*b*). A second objective in the present work was to compare the effect on flounder patterns of catecholamines which have been studied in other pleuronectid chromatophore systems without reference to patterning (Scott, 1965; Fernando & Grove, 1974*a*,*b*).

Control of flounder XA pigment migration has been described by Burton (1981). Although XA aggregation is neurally mediated the process is relatively slow during white-background adaptation, producing various cream, yellow and olive integumentary hues. XA dispersion in teleosts is under pituitary control (Chavin, 1956; Rasquin, 1958), and in flounder it is relatively rapid. High A and NA doses will aggregate flounder XA and, in the current work, this antagonism of the pituitary influence is further characterized.

MATERIALS AND METHODS

Winter flounder (150–500 g) were caught off the Avalon peninsula, Newfoundland, by Scuba divers with hand nets. Before experiments, flounders were kept in stock tanks supplied with running sea water (the temperature and illumination periodicity being seasonal) on local gravel and fed with caplin (*Mallotus mallotus*).

During experiments flounders were kept singly in rows of continuously illuminated (60 W, 1 m above) black or white Plexiglas aquaria (400 mm × 255 mm × 203 mm) supplied with running sea water through a header tank system. Aquaria were covered with wide-mesh nylon net on close-fitting wooden frames. Aquarium water temperatures were generally 4–12 °C with 1–2 °C variation during individual experiments, although one experiment (Table 1) was conducted at 15 °C.

L-Adrenalin bitartrate (Sigma), dopamine hydrochloride (Sigma), L-isoproterenol hydrochloride (ISOP) (Sigma), L-noradrenalin bitartrate (Sigma), phentolamine methanosulphate (PHA) (Ciba-Geigy), L-phenylephrine hydrochloride (PHE) (Sigma) and DL-propranalol hydrochloride (PRL) (Sigma) were injected subcutaneously, the vehicle being a balanced salt solution (BSS) (in mmol l^{-1}) (NaCl, 175; KCl, 27; $MgCl_2.6H_2O$, 0·64; $CaCl_2$, 1·53) based on analyses of plasma from locally caught winter flounder (Fletcher, 1977 and unpublished data). Doses are given as mol of drug base per kg of fish weight. Recovery intervals between individual CA injections were at least 48 h. Injections of flounder pituitary

extract used one pituitary equivalent per flounder, 0·1 mol l^{-1} HCl being the vehicle (Baker & Ball, 1975).

Surgical procedures were spinal section of the chromatic nerve fibres, adapted for this species (Burton, 1981), and hypophysectomy (Campbell & Idler, 1976). They were performed under tricaine methane sulphonate anaesthesia (Campbell & Idler, 1976).

Pattern responses to injections were recorded macroscopically. The method, using scale slip preparations for microscopically estimating the dermal and epidermal melanophore-index (DMI, EMI) and xanthophore index (XI), described by Burton (1981) was used to quantify the degree of pigment dispersion before injection and at suitable post-injection intervals. The means of three readings of each chromatophore index for each pattern component represented chromatophore index values for individual flounders. EMI values for iridophore spots were not included since the EPM are sparse or absent in the case of this pattern component. Tests of statistical significance of DMI, EMI and XI differences were made using the Mann-Whitney U test with the extended statistical tables of Rohlf & Sokal (1981).

RESULTS

Black-adapted flounders

The upper skin of black-adapted (> 3 day) flounders is predominantly black, dark grey or dark brown. Iridophore spots are white or pale grey, depending on the degree of dispersion of overlying dermal melanophores and xanthophores and the paucity of EPM. The bands are also usually discernible due to differences in chromatophore distribution (Burton, 1981).

Different doses of A, DA and NA were injected into separate groups of six black-adapted flounder. After each injection there were variable degrees of pallor of the general background and iridophore spot components within minutes; the dark bands, or large areas of them, remaining dark. One hour after the highest CA doses (A and NA, 5×10^{-6} mol kg^{-1}; DA, 5×10^{-5} mol kg^{-1}), pallor was relatively uniform, with only vestiges of the pattern discernible. With weaker doses, paling of the bands was refractory and they remained prominent 1 h after injection in most cases. They generally had the form typically associated with the normal response of black-adapted flounders to albedo change. Subsequent clearance of the injected CA was slow, pallor persisting 6 h or more after the highest A and NA doses and up to 2–3 h after lower doses. DA-induced pallor, evoked by higher doses than the A and NA, was not so persistent, lasting up to 3 h after the highest dose. Control injections, using the vehicle alone, did not elicit pallor.

Chromatophore 10 min and 1 h responses were quantified. With the lower doses aggregation was sometimes more advanced around the scale-slip periphery and chromatophore index determination was confined to a standard area within the centre and base of the skin sample. The differential aggregation of band and general background chromatophores 1 h after injection of NA is summarized in Fig. 1. The lowest dose (5×10^{-8} mol kg^{-1}) evoked aggregating responses in only three flounders, which were reverting after 10 min indicating relatively rapid NA clear-

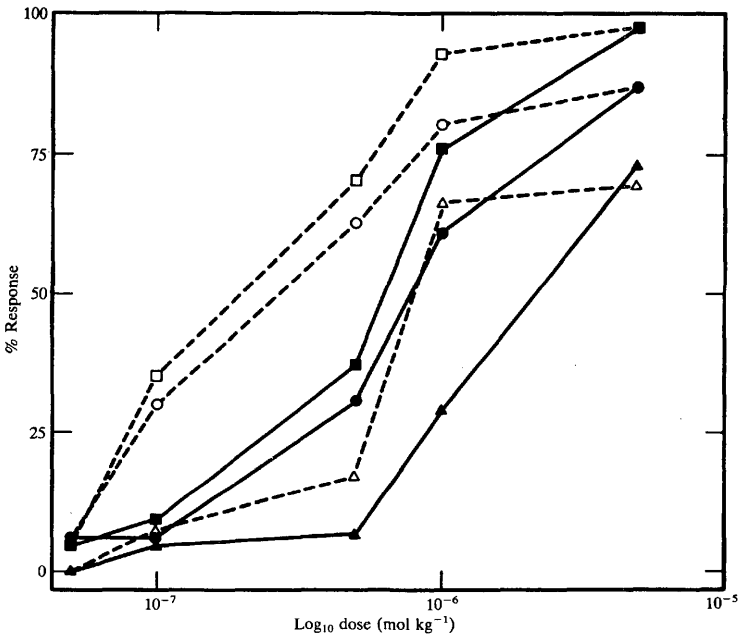

Fig. 1. Dark band (——) and general background (---) differential epidermal melanophore (■ □), dermal melanophore (● ○) and xanthophore (▲ △) aggregation in black-adapted flounders 1 h after injection of different noradrenalin doses. $N=6$ flounders. July–August; water temperature, 10–12°C.

ance. With doses $>10^{-7}$ mol kg^{-1}, 1 h aggregation exceeded that after 10 min. The maximum 1 h differences between band and general background melanophore aggregation were statistically significant (DM, $P<0{\cdot}025$; EPM, $P<0{\cdot}05$). Xanthophore aggregation was refractory with doses $\leq 5\times10^{-7}$ mol kg^{-1}. There was response heterogeneity with higher doses and the maximum differences between band and general background xanthophore aggregation was statistically only barely significant ($P\simeq0{\cdot}1$). Although, in general, there was iridophore spot DM and XA aggregation after NA injection the variability in the pre-injection condition made it difficult to quantify this response. Differential aggregation of the band and general background component chromatophores similar to that induced by NA and associated with albedo change also occurred with A and DA.

To investigate relative sensitivities to A, DA and NA, whilst minimizing the

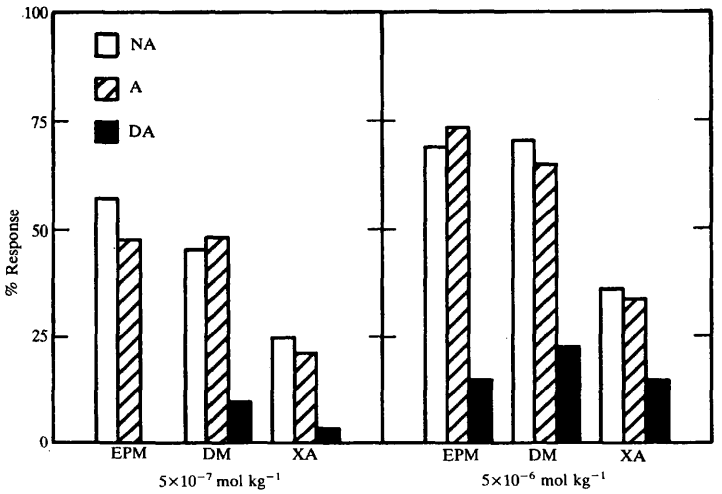

Fig. 2. Comparison of general background chromatophore aggregation 1 h after injection of a single group of flounders with different catecholamine compounds. $N=12$ flounders. May–June; water temperature, 4–5 °C. There was no measurable epidermal melanophore (EPM) response to the dopamine (DA) dose of 5×10^{-7} mol kg^{-1}. NA, noradrenalin; A, adrenalin; DA, dopamine; DM, dermal melanophore; XA, xanthophore.

effect of individual response variability, each flounder in a black-adapted group ($N=12$) was injected with each CA (5×10^{-7} mol kg^{-1}) in a specific sequence. Initially, a different CA was injected into each of three groups of four flounders, each group being evenly distributed between aquaria. Subsequent injection orders were rotated until each fish had received each CA in one of three sequences. The procedure was repeated for a 5×10^{-6} mol kg^{-1} dose. The percentage response is summarized in Fig. 2. There were no statistically significant differences ($P > 0{\cdot}1$) between chromatophore responses to A and NA. DA sensitivity was considerably lower than for A and NA, the differences being statistically significant (DM and EPM, $P < 0{\cdot}001$; XA, $P < 0{\cdot}025$) for each dose.

PHE (5×10^{-6} mol kg^{-1}), an α-adrenoceptor agonist in mammals, induced pigment aggregation (general background DM, EPM and XA responses being 49 %, 44 % and 31 % respectively, corresponding band responses being 21 %, 24 % and 15 %). The β-adrenoceptor agonist ISOP evoked little or no paling with a dose of 5×10^{-6} mol kg^{-1}, but there was pronounced uniform paling with doses from 10^{-5} to 5×10^{-5} mol kg^{-1}.

White-adapted flounders

After NA doses of 10^{-7} and 10^{-8} mol kg^{-1}, and with the vehicle (BSS) alone, there was band darkening in three out of six white-adapted flounders, but this did not occur with a dose of 10^{-6} mol kg^{-1}. Maximum increases in DMI and EMI

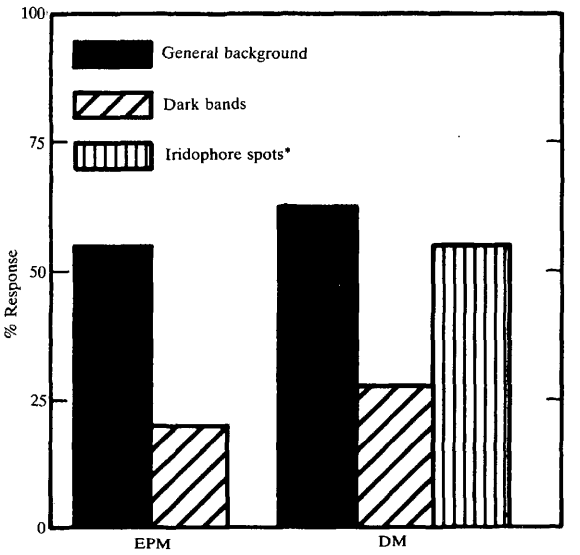

Fig. 3. Melanophore aggregation in chromatically spinal flounders 10 min after noradrenalin (5×10^{-7} mol kg^{-1}) injection. $N=6$ flounders. *Epidermal melanophores (EPM) not included (refer to Materials and Methods section). DM, dermal melanophores.

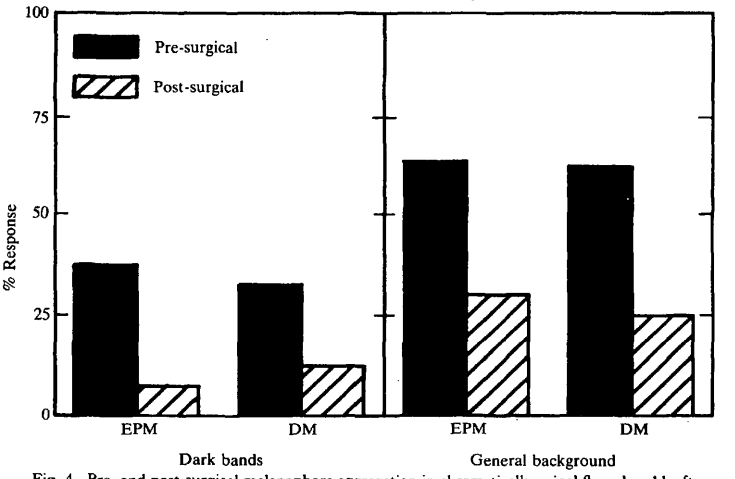

Fig. 4. Pre- and post-surgical melanophore aggregation in chromatically-spinal flounders 1 h after noradrenalin (5×10^{-7} mol kg^{-1}) injection. $N=6$ flounders, $P<0{\cdot}05$. EPM, epidermal melanophores; DM, dermal melanophores.

(from $1{\cdot}3 \pm 0{\cdot}1$ to $2{\cdot}4 \pm 0{\cdot}4$, and from $1{\cdot}2 \pm 0{\cdot}2$ to $2{\cdot}2 \pm 0{\cdot}5$, respectively) associated with the lower NA doses did not differ significantly ($P > 0{\cdot}1$) compared with DMI and EMI increases (from $1{\cdot}5 \pm 0{\cdot}1$ to $2{\cdot}2 \pm 0{\cdot}4$, and from $1{\cdot}4 \pm 0{\cdot}1$ to $2{\cdot}4 \pm 0{\cdot}4$, respectively) associated with the vehicle alone. It is likely that band darkening with low NA doses is the stress response (Burton, 1981) associated with handling flounders. ISOP (5×10^{-6}, 10^{-5} and 5×10^{-5} mol kg^{-1}) did not cause any general darkening of six white-adapted flounders, although there was transient (< 10 min) band darkening in four of these fish. However, control (vehicle) injection elicited similar, but more persistent (>30 min) band darkening in all six flounders.

Chromatically spinal flounders

The black-adapted flounders ($N=6$) used for the pattern responses to NA (Fig. 1) were later chromatically decentralized by spinal section anterior to vertebra 5 (Burton, 1981), and injected with NA (5×10^{-7} mol kg^{-1}) 3 days after surgery. Melanophores and xanthophores in spinal flounders are highly dispersed and, in general, NA evoked differential paling of the pattern components similar to the pre-surgical response. The differences between post-surgical band and general background melanophore aggregation (Fig. 3) were statistically significant ($P < 0{\cdot}005$). Post-surgical iridophore spot data were particularly interesting since the DM initial dispersion was more homogeneous than in intact flounders. NA-induced aggregation of these DM (Fig. 3) was not significantly different from that of general background DM. Post-surgical XA responses to NA were as heterogeneous as in intact flounder, and there were no statistically significant differences between pattern components. Post-surgical NA-induced pallor was not as persistent as that before surgery and darkening was appreciable within 1 h. Thus, 1 h after NA injection, spinal fish band and general background melanosomes were significantly ($P < 0{\cdot}05$) more dispersed than in their pre-surgical response (Fig. 4). There were no significant differences between XA pre- and post-surgical responses.

Effects of adrenoceptor antagonists

Phentolamine, an α-adrenergic antagonist in mammals, induced uniform darkening of white-adapted flounders with extensive DM, EMP and XA dispersion (Table 1). In hypophysectomized-spinal flounders the XA are highly aggregated (Table 2) and PHA did not elicit the extensive XA dispersion which it evoked in white-adapted intact flounder. Flounder pituitary extract produced extensive XA dispersion in hypophysectomized, spinal flounder (Table 2) consistent with strong pituitary control of this process. Pallor, and associated DM, EPM and XA aggregation ($P < 0{\cdot}025$), induced by the β-adrenergic agonist ISOP (Table 3) was inhibited by an equimolar PHA dose.

The β-adrenoceptor antagonist PRL (5×10^{-6} and 10^{-5} mol kg^{-1}) did not elicit general pallor of black-adapted flounder ($N=4$), but a $2{\cdot}5 \times 10^{-5}$ mol kg^{-1} dose resulted in partial paling of the general background component, but not of the bands. These PRL doses did not darken white-adapted flounders ($N=4$). The highest dose approximated the toleration limit to PRL since three flounders ($N=8$) exhibited abnormal degrees of swimming and one died 6 h after injection.

Table 1. *Pigment dispersion induced by PHA*

Component and time		Chromatophore index EMI	DMI	XI
DB	Pre	1·1 ± 0·1	1·4 ± 0·2	2·9 ± 0·4
	1 h	4·9 ± 0·1	4·8 ± 0·1	5·0
GB	Pre	1·0	1·2 ± 0·1	2·2 ± 0·5
	1 h	4·4 ± 0·3	4·2 ± 0·5	4·8 ± 0·1
IS	Pre	*	1·0	1·2 ± 0·1
	1 h	*	4·2 ± 0·7	4·8 ± 0·2

Mean ± S.E.M. values are for five flounders. PHA, $2{\cdot}5 \times 10^{-5}$ mol kg^{-1}.
DB, dark band component; GB, general background component; IS, iridophore spot component. Pre, pre-injection. *See Materials and Methods.

Table 2. *Xanthophore pigment dispersion induced by PHA and by pituitary extract*

Treatment	*N*	Time	Xanthophore index DB	GB	IS
Intact, white-adapted + PHA	5	Pre	1·5 ± 0·3	1·2 ± 0·1	1·0
		1 h	4·9 ± 0·7	4·3 ± 0·3	3·6 ± 0·5
Hypophysectomized, spinal + PHA	6	Pre	1·4 ± 0·2	1·5 ± 0·2	1·1 ± 0·1
		1 h	2·1 ± 0·2	1·8 ± 0·2	1·4 ± 0·2
Hypophysectomized, spinal + pituitary extract	8	Pre	1·9 ± 0·3	1·4 ± 0·3	1·3 ± 0·2
		1 h	5·0	4·6 ± 0·2	3·2 ± 0·5

XI values = means ± S.E.M. PHA, $2{\cdot}5 \times 10^{-5}$ mol kg^{-1}.
Pituitary extract = 1 pituitary equivalent.
DB, dark band component; GB, general background component; IS, iridophore spot component; Pre, pre-injection.

Table 3. *Chromatophore responses to ISOP ($2{\cdot}5 \times 10^{-5}$ mol kg^{-1}) of general background component of flounder, previously darkened by PHA ($2{\cdot}5 \times 10^{-5}$ mol kg^{-1}) or by black-background adaptation*

	PHA darkened* EMI	DMI	XI	Black-background darkened EMI	DMI	XI
Before ISOP injection	4·2±0·3	4·7±0·2	4·8±0·2	5·0	4·8±0·1	4·5±0·3
1 h after ISOP injection	4·1±0·4	4·8±0·1	4·8±0·2	3·3±0·3	3·1±0·1	3·2±0·3

Mean ± S.E.M. values are for six flounders.
*White-adapted flounders, PHA injection 1 h before ISOP injection.

DISCUSSION

Interpretation of data from injected, plasma borne, neurotransmitters can be complicated by possible involvement of extraneous structures, including neuronal and circulatory systems. However, there are significant differences in CA sensitivity between flounder pattern components. Moreover, patterning elicited by A, DA and NA largely simulates that associated with albedo change after transferring black-adapted flounders to a white background. The lower catecholaminergic sensitivity of band melanophores is consistent with their refractoriness during aggregation on a white background, and also with their considerable capacity for rapid dispersion following transfer to a black background. This implicates a neurally-mediated melanosome dispersing mechanism in the bands, which is antagonistic to injected catecholamines. This conclusion is further supported by the stress-related capacity of band chromatophores to override aggregation induced by all except the highest NA doses. Teleost neural-chromatophore systems may include post-ganglionic cholinergic dispersing neurones (Parker, 1948). Recent *in vitro* studies implicate α-adrenoceptors in melanosome aggregation and β-adrenoceptors in dispersion in *Lebistes reticulatus* (Fujii & Miyashita, 1975; Miyashita & Fujii, 1975) and cholinoceptors and adrenoceptors in melanosome dispersion in *Bathygobius soporator* (Visconti & Castrucci, 1981).

Supersensitivity to NA and stress associated with chronic spinal lesion (Grove, 1969; Burton, 1981) was not a characteristic of flounders only 3 days after spinal section. No reference could be found to spinal section shortening the duration of CA-induced pallor in teleosts, which may be the result of chromatophore de-sensitization or of an indirect physiological effect. The indirect effects of spinal section on chromatophores are unknown.

Flounder chromatophore *in vivo* responsiveness to A and NA was greater than that to DA and ISOP. For pharmacokinetic reasons, and the possible involvement of other systems, these *in vivo* relative sensitivities may not necessarily reflect the direct effectiveness of individual CA compounds on the chromatophores. *In vitro* studies may clarify this issue, whilst actual identification of the neurotransmitter will require chemical characterization *in situ*. Nevertheless, the present results contrast with *Pleuronectes*, in which DA is more potent than A or NA in inducing pallor (Fernando & Grove, 1974*a*,*b*), and with the extreme effectiveness of all three compounds in *Scophthalamus* (Scott, 1965) and *Phoxinus* (Healey & Ross, 1966). These differences may be associated with physiological adaptation to different temperature ranges. It is pertinent to note that more extensive mammalian studies have not produced compelling evidence for peripheral dopaminergic neurones (Burnstock & Costa, 1975).

Inhibition of melanosome aggregation by PHA conforms with the view that teleost melanosome aggregation has an α-adrenergic character (Falck, Muntzing & Rosengren, 1969; Fernando & Grove, 1974*a*,*b*; Fujii & Miyashita, 1975), but the involvement of different pattern components represents new information. The effect of PHA on XA aggregation is particularly interesting since little is known about xanthophore control. The ineffectiveness of PHA in eliciting xanthophore dispersion in the absence of a pituitary factor supports the view (Burton, 1981) that

XA dispersion is an active process rather than a 'passive' process. Thus XA aggregation is both endogenous and a result of CA antagonism of the pituitary pigment-dispersing factor. The pituitary influence accounts for the refractoriness of both background-related XA aggregation and response to low CA doses and, probably, for XA response variability.

β-Adrenoceptor mediation of melanosome dispersion has been suggested (Reed & Finnin, 1972; Miyashita & Fujii, 1975; Obika, 1976) in some teleosts, and Komatsu & Yomada (1982) have demonstrated, by autoradiography, uniformly distributed β-adrenoceptors on melanophores of *Oryzias latipes*. ISOP evokes transient melanosome dispersion in *Pterophyllum eimekei* (Reed & Finnin, 1972). Absence of ISOP-related darkening in flounder suggests that melanosome dispersion does not have a β-adrenoceptor character in this species. ISOP related melanosome aggregation in flounder is also characteristic of other teleosts (Scott, 1965; Healey & Ross, 1966; Grove, 1969; Fernando & Grove, 1974*a*,*b*; Fujii & Miyashita, 1975). PHA antagonism of ISOP is not consistent with a β-adrenoceptor character in the case of flounder melanosome aggregation. At the high doses used ISOP could have an additional α-adrenoceptor action, as in mammals (Innes & Nickerson, 1975). Alternatively, ISOP may indirectly involve effector α-adrenoceptors through stimulation of aggregating neurones sensitive to β-adrenoceptor agonists. *In vitro* the β-adrenergic antagonist PRL does not disperse *Pleuronectes* aggregated melanophores (Fernando & Grove, 1974*b*), but it inhibits A-induced (low dose) dispersion in tolazaline treated melanophores of *Lebistes* (Miyashita & Fujii, 1975). Flounder did not tolerate PRL well at doses eliciting partial pallor which is probably non-specific. For example, PRL may reduce erythrocytic oxygen affinity (Oski *et al.* 1972; Pendleton *et al.* 1972) indirectly influencing chromatophores, at the high doses used.

The differential responses of flounder pattern components to CA compounds is supportive of a patterning mechanism largely dependent on a balance between neural aggregating and dispersing elements. Possible differences in innervation, neurotransmitter release, receptor type and number could provide the basis for the patterning mechanism. Based on mammalian criteria, the present study suggests that flounder chromatophore adrenoceptors have an α-adrenergic character. However, Visconti & Castrucci (1981) have described teleost melanophore adrenoceptors which are uncharacterized with respect to α and β nature, and it may be necessary to reassess current views on teleost melanophore receptors. Characterization of adrenoceptors, possible cholinoceptors and pituitary receptors in flounder pattern components would be best undertaken *in vitro* and further research is being directed towards this goal.

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REFERENCES

BAGNARA, J. I. & HADLEY, M. E. (1973). *Chromatophores and Color Change*, pp. 109–112. Englewood Cliffs: N. J.: Prentice Hall.

BAKER, B. I. & BALL, J. N. (1975). Evidence for a dual pituitary control of teleost melanophores. *Gen. comp. Endocr.* **25**, 147–152.

BURNSTOCK, G. & COSTA, M. (1975). *Adrenergic Neurons, Their Organisation, Function and Development in the Peripheral Nervous System*, p. 5. London: Chapman & Hall.

BURTON, D. (1981). Physiological responses of melanophores and xanthophores of hypophysectomized and spinal winter flounder, *Pseudopleuronectes americanus* Walbaum. *Proc. R. Soc. B* **213**, 217–231.

CAMPBELL, C. M. & IDLER, D. R. (1976). Hormonal control of vitellogenesis in hypophysectomized winter flounder (*Pseudopleuronectes americanus*, Walbaum). *Gen. comp. Endocr.* **28**, 143–150.

CHAVIN, W. (1956). Pituitary-adrenal control of melanization in xanthic goldfish, *Carassius auratus* L. *J. exp. Zool.* **133**, 1–36.

FALCK, B., MUNTZING, J. & ROSENGREN, A. M. (1969). Adrenergic nerves to dermal melanophores of the rainbow trout, *Salmo gairdneri*. *Z. Zellforsch. mikrosk. Anat.* **99**, 430–434.

FERNANDO, M. M. & GROVE, D. J. (1974*a*). Melanophore aggregation in the plaice (*Pleuronectes platessa* L.). I. Changes in *in vivo* sensitivity to sympathomimetic amines. *Comp. Biochem. Physiol.* **48**A, 711–721.

FERNANDO, M. M. & GROVE, D. J. (1974*b*). Melanophore aggregation in the plaice (*Pleuronectes platessa* L.). II. *In vitro* effects of adrenergic drugs. *Comp. Biochem. Physiol.* **48**A, 723–732.

FLETCHER, G. L. (1977). Circannual cycles of blood plasma freezing point and Na^+ Cl^- concentrations in Newfoundland winter flounder (*Pseudopleuronectes americanus*): correlation with water temperature and photoperiod. *Can. J. Zool.* **55**, 789–795.

FUJII, R. & MIYASHITA, Y. (1975). Receptor mechanisms in fish chromatophores. I. Alpha nature of adrenoceptors mediating melanosome aggregation in guppy melanophores. *Comp. Biochem. Physiol.* **29**, 109–126.

GROVE, D. J. (1969). The effects of adrenergic drugs on the melanophores of the minnow, *Phoxinus phoxinus*. *Comp. Biochem. Physiol.* **19**, 545–580.

HEALEY, E. G. & ROSS, D. M. (1966). The effects of drugs on the background response of the minnow, *Phoxinus phoxinus* L. *Comp. Biochem. Physiol.* **19**, 545–580.

INNES, I. R. & NICKERSON, M. (1975). Norepinephrine, epinephrine and the sympathomimetic amines. In *The Pharmacological Basis of Therapeutics*, (eds L. S. Goodman & A. Gilman), pp. 477–513. New York: MacMillan Publishing Co.

JACOBOWITZ, D. M. & LATIES, A. M. (1968). Direct adrenergic innervation of a teleost melanophore. *Anat. Rec.* **162**, 501–504.

KOMATSU, K. & YAMADA, K. (1982). Autoradiographic visualization of the beta adrenergic receptors in fish melanophores. *J. exp. Zool.* **223**, 185–187.

MIYASHITA, Y. & FUJII, R. (1975). Receptor mechanisms in fish chromatophores. II. Evidence for beta adrenoceptors mediating melanosome dispersion in guppy melanophores. *Comp. Biochem. Physiol.* **51**C, 179–187.

OBIKA, M. (1976). An analysis of the mechanism of pigment migration in fish melanophores. *Pigment Cell* **3**, 254–264.

OSKI, F. A., MILLER, L. D., DELIVORIA-PAPADOPOULOS, M., MANCHESTER, J. H. & SHELBURN, J. C. (1972). Oxygen affinity in red cells: changes induced *in vivo* by propranalol. *Science, N.Y.* **175**, 1372–1373.

PARKER, G. H. (1948). *Animal Colour Changes and Their Neurohumors*, pp. 144–154. Cambridge: Cambridge University Press.

PENDELTON, R. G., NEWMAN, D. J., SHERMAN, S. S., BRANN, E. G. & MAYA, W. E. (1972). Effect of propranalol upon the hemoglobin-oxygen dissociation curve. *J. Pharmac. exp. Ther.* **180**, 647–656.

RASQUIN, P. (1958). Studies on the control of pigment cells and light reactions in recent teleost fishes. *Bull. Am. Mus. nat. Hist.* **115**, 1–68.

REED, B. L. & FINNIN, B. C. (1972). Adrenergic innervation of melanophores in a teleost fish. In *Pigmentation: Its Genesis and Biologic Control*, (ed. V. Riley), pp. 285–294. New York: Appleton, Century, Crofts.

ROHLF, F. J. & SOKAL, R. R. (1981). *Statistical Tables* 2nd Edition, pp. 185–190. San Francisco: W. H. Freeman.

SCOTT, G. T. (1965). Physiology and pharmacology of color change in the sand flounder. *Limnol. Oceanogr. (suppl.)*, **10**, R230–R246.

VISCONTI, M. A. & CASTRUCCI, A. M. (1981). Evidence of adrenergic and cholinergic receptors in teleost melanophores. *Comp. Biochem. Physiol.* **70**C, 293–296.